Critical Parameters for Predictive Modeling of Chemical Agent Persistence on the Battlefield: The Effective Half-Life of VX on Plant Foliage

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Abstract

Methods for investigating Chemical Warfare Agent (CWA)–Plant Interactions have successfully been developed using live plant species, necessary in order to maintain plant physiological responses and obtain results applicable to CWA-contaminated battlefields. Pioneering research included sustaining living plants within mandatory surety hood constraints, and disseminating CWA onto individual mature leaves of the foliage of intact living plants. The CWA selected for method development and initial research is VX [O-Ethyl-S-(2-diisoproylaminoethyl) methyl phosphono thiolate]. The plant species selected for method development and initial research is the grass *Echinochloa crus-galli*. Grass is the most prevalent type of higher plant worldwide, and the natural distribution of *Echinochloa crus-galli* is one of the largest. Traditional plant culture under controlled environmental conditions outside of surety hoods typically involves balancing heat loads with large chilling units too cumbersome for most surety hoods. Because physiologically healthy living plants are required in order to investigate and record critical parameters for the effects of CWA–Plant Interaction, we installed and tested a system of light-emitting diodes (LED) within the surety hood in order to supply high-quality photosynthetically active radiation (PAR). Environmental conditions within the surety hood were: illumination >250 umoles cm^2 sec^{-1}, 16h-light/8h-dark; 21°C; relative humidity >45%. The experimentally determined Effective Half-Life of VX on grass leaves is 72h (95% CI 46-98), calculated using the statistical Logistic Gompertz Model which provided the best fit to observed experimental data. Effective Half-Life is a measure of the net effect of all factors affecting CWA persistence, including evaporation, transformation, and fixation. Additional critical parameters under investigation include the coefficient of wash-off from measured rainfall, distribution of CWA on and within leaves as a function of time, and Contact Transfer (exposure) of CWA from contaminated foliar surfaces onto Army Combat Uniform (ACU). Results of these investigations provide critical parameter input for predictive models, direct experimental determinations for comparison of predictive model outcomes, plus information for decision-making affecting Soldiers on CWA contaminated battlefields.

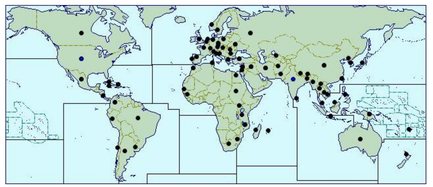
Background

- **Little information existed on the interactions of CWA with plants.**
- **New methods needed to be developed for sustaining plant culture in a surety hood environment, in order to investigate CWA–Plant Interactions using healthy live plant species with phytophysiological responses applicable to contaminated battlefields.**
- **CWA on foliage has to be within an appropriate range (e.g., battlefield), yet be detectable.**

Approved for Public Release

Materials & Methods

Plant species:
***Echinochloa crus-galli* (L.), P. Beauv.; Grass is the most prevalent type of higher plant worldwide, and the natural distribution of *Echinochloa crus-galli* is one of the largest worldwide**



← **Each dot represents a country where the grass *Echinochloa crus-galli* is commonly found**

Plant Physiology: Mature leaf stage

CWA: VX [O-Ethyl-S-(2-diisoproyl aminoethyl) methyl phosphono thiolate]

Dissemination: 1µL VX droplet (near optimal size-range for materiel testing) onto the surface of individual mature leaf, horizontally stabilized; randomly selected mature plant leaves received treatment; quadruplicate replication

Sample Times: Individual leaves that received VX, and control leaves, were collected at 0.017, 1, 4, 24, 48, 120, 168 hours after VX dissemination

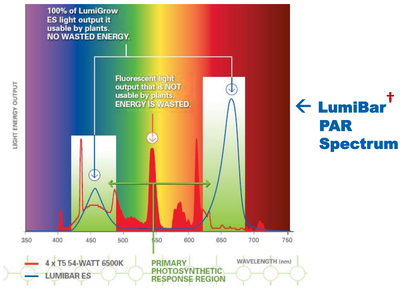
Plant culture: (pre-surety hood) Twenty seeds are sown in potting mix, and hydrated with ASTM Type I water (18 MΩ cm); following germination (80-95% after 7-10 days), individual grass plants are transplanted into 10cm containers and randomized within an environment-controlled plant growth chamber; Plants thereafter receive dilute aqueous nutrient solution every 2-3 days to maintain moisture (by mass) and sustain healthy plants. Chamber conditions: 22°C ±2 (16h-light) and 18°C ±2 (8h-dark); relative humidity 60% ±5; illumination 300-350 µmol s^{-1} m^{-2} photosynthetically active radiation (PAR; 400-700ηm)

Plant culture: (in surety hood) Plants with 2-3 fully-mature leaves (18-21 days after transplantation) are transferred into Surety Hood conditions: 21°C ±2; 16h-light and 8h-dark; relative humidity ≥45%; illumination 300-350 µmol s^{-1} m^{-2} PAR delivered using LumiGrow LumiBar LED Strip Lighting†

† Mention of a product or Trade Name does not constitute endorsement

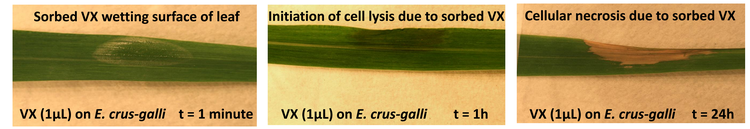
Results

Echinochloa crus-galli growing in Surety Hood



← **LumiBar† PAR Spectrum**

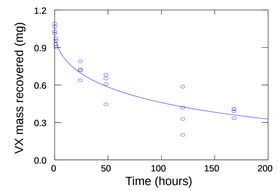
VX on leaf: Upon dissemination, VX spread and sorbed onto and into live mature grass leaves, wetting the leaf surface; After 1 hour, the area where VX sorbed appeared darkened apparently due to initiation of cell lysis, then necrosis:



Leaf Analysis: Treated and control leaves are removed from plants, 14cm from the leaf tip, then prepared and analyzed, by:

1) Placing each leaf into a Mylar† sample bag attached to a test tube
2) Flash-freezing in liquid N_2
3) Pulverizing leaf in Cryo-Prep Impactor†
4) Adding extraction solvent, isopropyl alcohol
5) Focused Ultra-Sonication
6) Centrifugation
7) Analytically determining VX by UHPLC-Triple Quad MS

Data Analysis: The Logistic Gompertz Model provided the best fit to the observed experimental data



VX Mass Recovered From Contaminated Grass Leaves As a Function of Time Post-Dissemination

The Effective Half-Life‡ of VX on Grass (*Echinochloa crus-galli*) was experimentally determined to be: 72 hours
95% confidence interval 46-98

‡ The Effective Half-Life is a measure, as a function of time post-dissemination, of the net effect of all factors affecting the persistence of CWA, including evaporation, transformation, and fixation

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